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[54] Title
DRUG DELIVERY SYSTEM AND METHOD OF MAKING THE SAME.

[57] Abstract
(From EP 302582 A) A compatible, biodegradable microcapsule delivery system for active ingredients, including hormonally active peptides, proteins, or other bioactive molecules, and a method of making the same. The ingredients are encapsulated in biodegradable copolymer excipients of varying mole ratios and the blend of the microcapsules are administered to an animal.

Delivery of the ingredient occurs over a prolonged period of time at a constant rate as a result of the varying break-down rates of the copolymer excipients.

This invention relates to a composition capable of delivering an effective amount of a constant dose of bioactive molecule at a constant rate and in particular to a drug delivery composition.

It is known that a marked inhibition of pituitary and gonadal function that occurs after chronic adminstration of the [D-Trp ,des-Gly]-LHRH ethylamide an analog of luteinizing hormone releasing hormone (LHRH) and other LHRH analogs leads to a reduction in steroidal sex hormones and makes possible approaches for the use as a contraceptive or for the treatment of sex hormone-dependent tumours. Concerning the latter, studies involving rats treated with LHRH analogs show the potential clinical efficacy of the hormone in the treatment of prostrate carcinoma and other hormone-dependent tumours in animals.

The treatment of hormone-dependent tumours and other disorders in animals would be greatly enhanced by a delivery system which, after a single administration, maintained controlled levels of active ingredients, including [D-Trp ,des-Gly]-LHRH ethylamide and its related analogs, over extended periods of time. Traditional methods of administering peptides (or proteins) result in high initial concentrations of peptide (or protein) analog in the tissue, but over a short
period of time, i.e., over a few minutes to several hours, peptide levels in the blood decline. Therefore, optimal pharmacological effects are most often not achieved. The result is a need for more frequent adminstration of higher-dosage regimens.

More recently, a polymer of poly(D,L-lactide-co-glycolide) (DL-PLG), which is biodegradable and biocompatible with living tissue, has been used in microcapsules for longer acting delivery systems. Systems of microencapsulated active ingredients in polymers and copolymers of lactic acid and glycolic acid have been used to achieve controlled release of chemical and biological pharmaceuticals. For example, U.S. Patent No. 3,773,919 discloses a drug, stated to include water-soluble antibiotic peptides encapsulated in lactide/glycolide copolymers so as to provide controlled release. Canadian Patent No.

1,176,565 discloses a microcapsule composition comprising a core containing a LHRH peptide encapsulated in a biodegradable, biocompatible copolymer excipient.

Microencapsulation for controlled release of enzymes, hormones and other biologicals are discussed in papers by Sanders, Kent, McRae, Vickery, Tice, and Lewis,
Journal of Pharmaceutical Sciences, Vol. 73, pp. 1294-1296, September 1984 and by Redding, Schally, Tice and Meyers, Proc. Natl. Acad. Sci. USA, Vol. 81, pp. 5845-5848, September 1984. The first paper describes a system controlled by diffusion and erosion, wherein the kinetics of compound release determined by the parameters of the copolymer, and more particularly, the controlled release of nafarelin acetate, an analog of LHRH, from poly(D,L-lactide-co-glycolide) microspheres. The second paper discloses the inhibition of rat prostrate tumours by controlled release of [D-Trp] luteinizing hormone-releasing hormone from injectable microcapsules.

The microcapsule systems described in the above-publications all share a common feature in that the release of the compound is controlled by the porosity and/or erosion of a polymer continuum. Also, all the described microcapsule systems utilize only a single type of copolymer. Therefore, while a controlled release of the compound is achieved, such is limited by the specific lactide/glyoclide ratio used in the encapsulating material. At the most, the methods previously used, and particularly the peptide microcapsule, provided release times of approximately one month.

WO 87/06129 discloses a sustained release implant comprising a plurality of biodegradable microcapsules containing a physiologically active ingredient, the microcapsules being embedded in a biodegradable polymeric article. This document does not disclose how to produce sustained release of the bioactive ingredient at a constant rate.

There exists, therefore, a need for a method of delivering active ingredients, including peptides, proteins and other bioactive molecules used in treating disease, which utilize the advantages of microencapsulation, but which provides a longer controlled duration of release than that presently known. Also, there exists a need for a method of

providing a constant dose regime of active ingredient throughout the longer release time provided by using biodegradable microcapsules.

According to the present invention there is provided a composition capable of delivering an effective amount of a constant dose of bio-active molecule at a constant rate to an animal over a preselected, prolonged period of time, comprising a blend of free-flowing microcapsules in which effective amounts of a bioactive molecule are encapsulated in at least two biodegradable and biocompatible copolymer excipients to form first and second microcapsules, each excipient capable of a different rate of release of said molecule therethrough, said composition having a delivery profile wherein the release of said molecule through said second microcapsule begins as the release of said ingredient through said first microcapsule declines. This allows the delivery of an active ingredient into the system of an animal at a constant rate over a long period of time, i.e one and one-half to six months or longer. Preferably, the composition comprises a blend of free flowing spherical particles and an effective amount of the microcapsule blend may be administered to the animal parenterally (e.g intravenously, intramuscularly, subcutaneously, intranasally, intraperitoneally, or by inhalation).

A quantity of these particles are of such a copolymer excipient that the core active ingredient is released quickly after injection, and thereby delivers the ingredient for an initial period. A second quantity of the particles are of such type excipient that delivery of the encapsulated ingredient begins as the first quantity's delivery begins to decline. A third quantity of ingredient may be encapsulated with a still different excipient which results in delivery beginning as the delivery of the second quantity begins to decline. Obviously, still greater assortments of excipients can be used to obtain more prolonged release time of the encapsulated ingredient. A further modification of the present invention could be to have different ingredients encapsulated within a blend of varying excipient formulations.

It is shown, therefore, that as the usefulness of one type of particle begins to decline or run out, another type begins to take over. This provides a preselected, constant rate of delivery over a prolonged period of time. For example, by varying the lactide/glycolide ratio in a

poly(D,L-lactide-co-glycolide) encapsulation, as well as the types and quantities of encapsulated active ingredient, it is possible to design a long-term, controlled-release profile of choice.
More particularly, the invention relates to a compatible, biodegradable, injectable microcapsule delivery system for the peptide agonist [D-Trp ,des-Gly]-LHRH ethylamide (hereinafter referred to as the "agonist") and for the peptide antagonist [D-N-Ac-4-Cl-Phe^2,D-Trp ,D-Ala]-LHRH (or an LHRH antagonist of similar structure) (hereinafter referred to as the "antagonist"). The microcapsule formation consists of free-flowing spherical particles, preferably of poly(D,L-lactide-co-flycolide) which can be administered parenterally, (e.g intravenously, intramuscularly, subcutaneously, intranasally, intraperitoneally or by inhalation). By utilizing a combination of various polymers with different lactide/glycolide ratios, one can greatly prolong the release profile of the encapsulated LHRH analog. Delivery periods of six months or more can be achieved.

In one aspect of the invention the biocompatible microcapsule delivery system is for the agonist [D-Trp ,des-Gly]-LHRH ethylamide which delivers the agonist at a constant rate of 50 μg to 250 μg or more per day for a duration of from one and one-half to six months or more in men and women.

In another aspect of the invention the biocompatible, biodegradable microcapsule delivery system is for the antagonist [D-N-Ac-4-Cl-Phe^2,D-Trp ,D-Ala]-LHRH, or an LHRH antagonist of similar structure, which delivers that antagonist at a constant rate of 200 μg to 500 μg or more per day for a duration of from one to three months or more.

An illustration of the method of preforming one embodiment of the invention, that is, the use of LHRH agonist encapsulated in poly (D,L-lactide-co-glycolide), follows. In addition, the details and results of a study utilizing this embodiment in rats are provided.

It should be noted, that other polymers besides poly(D,L-lactide-co-glycolide) may be used. Examples of such polymers include, but are not limited to: polyacetal polymers, polyorthoesters, polyesteramides, polycaprolactone and copolymers thereof,

polycarbonates, polyhydroxybutyrate and copolymers thereof, polymaleamides, copolyoxalates and polysaccharides.

I. PREPARATION OF DL-PLG EXCIPIENTS

The general procedures used to prepare DL-PLG copolymers and the results of their characterization are detailed in the following sections.

a. DL-Lactide Purification

DL-lactide was used to prepare the polymers. To purify the monomer, it is first dissolved by heating a mixture of the monomer in a volume of dry (stored over molecular sieves) ethyl acetate about equal to its weight. While still hot, the solution is vacuum filtered through an extra coarse, fitted-glass gas-dispersion tube. The solvent level is reduced with an aspirator to a level equal to about half the weight of the lactide. The solution is then allowed to cool slowly to room temperature and chilled in an ice-water bath to effect crystallization. The monomer is finally filtered in a nitrogen-filled glove box. The monomer is recrystallized from ethyl acetate two additional times in this manner. All glassware used after the initial hot filtration and recrystallization is oven dried overnight at 150°C prior to use. After the final recrystallization, the purified monomer is vacuum dried in a desiccator and stored in oven-dried glass jars until ready for use.

b. Glycolide Synthesis and Purification

The glycolide monomer is prepared and purified by the following method: Excess water is first distilled from 67% aqueous glycolic acid (Eastman Chemicals, Rochester, N.Y.) in a 3-neck flask equipped with a thermometer, distillation head, and a condenser. The solution is
boiled at reduced pressure with the use of a water aspirator. After the excess water has evolved, heating is continued to remove additional water by dehydration of the glycolic acid. After no further water is evolved, the flask is allowed to cool to room temperature under vacuum. At this point, about 1 percent by weight of antimony oxide, based on the theoretical glycolic acid content, is added to the flask as a catalyst. The distillation head and condenser are removed, and the flask is connected to

two receiving flasks and a trap arranged in series. The receiving flasks and trap are cooled by dry-ice: isopropanol baths. (Note: The first receiving flask is for product collection. The second receiving flask is actually a trap). The pressure is reduced to about 266 N/m^2 (mmHg), and the reaction flask is heated to distil the crude glycolide. The material that distils between 110 and 130°C is collected in the first receiving flask.

The crude glycolide collected is next purified by first washing the product. This is achieved by slurrying the glycolide in isopropanol, followed by filtering and vacuum drying, and then by three recrystallizations from ethyl acetate. After washing, precautions are made to protect the glycolide from atmospheric moisture during each stage of recrystallization by using oven-dried glassware, dry ethyl acetate (stored over molecular sieves), and a glove box filled with nitrogen. The crude glycolide is combined with a volume of ethyl acetate approximately equal to three-fourths its weight. The mixture is then heated to reflux to dissolve the glycolide and cooled slowly to room temperature to allow crystallization. The monomer is recrystallized three times in this manner. After each recrystallization, the glycolide crystals are collected by vacuum filtration in a glove box. After the final recrystallization, the product is dried at room temperature under a vacuum of <266N/m^2 (<2 mmHg) in a desiccator. The purified dried monomer is then stored in oven-dried glass jars placed inside a desiccator.

c. Copolymer Synthesis

All glassware is oven dried at 150°C overnight and allowed to cool in a nitrogen-filled glove box. All handling of the reactants and assembling of apparatus is done in the glove box. The purified monomers are weighed directly into a 3-neck, round-bottom flask. After being charged and sealed, the flask assembly is evacuated three times, back filled with nitrogen, removed from the glove box, connected to a dry nitrogen purge, and placed into an oil bath maintained at 170°C. Once the monomers have partially melted, stirring is begun. Positive nitrogen pressure is maintained over the monomers throughout the polymerization. After the monomers have completely melted, 0.05 percent by weight of stannous octoate is introduced into the flask with a microsyringe. Stirring is continued until the mixture becomes too viscous to

stir, at which point the stirrer is raised out of the melt. The polymerization is then continued for a total reaction time to 16 to h. Next, the resulting polymer is allowed to cool to room temperature under nitrogen atmosphere and removed by breaking the flask. Any residual glass is removed from the polymer plug by submerging it into liquid nitrogen. While cold, the polymer is broken into several smaller pieces and dissolved in methylene chloride and precipitated into methanol. The solvent is then removed by evaporation at room temperature under a hood and, finally, under vacuum at <266 N/m² (<2 mmHg)and about 40°C. The yields are typically about 75% of theoretical. The polymers are then characterised and stored in a desiccator until ready for use.

II. PREPARATION AND CHARACTERIZATION OF AGONIST LHRH MICROCAPSULES

The phase-separation microencapsulation process is used in this example to prepare microcapsules with the LHRH agonist and DL-PLG excipients. DL-PLG is dissolved in methylene chloride and placed in a resin kettle equipped with a true-bore stirrer that is fitted with a

3.75cm. Teflon turbine impeller and powered by a Fisher Stedi-speed stirrer at a speed of about 3000 rpm. The peptide is then dispersed in the stirrer copolymer solution followed by the addition of silicone oil (Dow 200 Fluid, 3.5x10 m^2/s (350 cSt), Dow Corning Corp., Midland, MI) to the resin kettle. This silicone oil causes the DL-PLG to coacervate and deposit onto the peptide particles. Immediately after the silicone addition is complete, the contents of the resin kettle are poured into 2 l of heptane being stirred at about 800 rpm with a 5 cm (2 in.) stainless steel impeller. The heptane causes the microcapsules to harden by extracting methylene chloride out of the microcapsules. After the stirring is continued for 30 min., the hard microcapsules are isolated by filtration and dried for 24 hours in a vacuum desiccator maintained at room temperature.

The core loading of the microcapsules is a measure of the amount of LHRH incorporated inside the microcapsules. This analysis is based on the extraction of core material (LHRH) from a known amount of microcapsules and quantification of the extracted LHRH by high performance liquid chromatography. A known amount of microcapsules is dissolved in methylene chloride. The LHRH is then extracted into triethylammonium phosphate (TEAP) buffer (pH 2.5) and

is injected into an HPLC for quantification.

The theoretical core loading for a batch of microcapsules is based upon the copolymer and LHRH input and is calculated in the following manner:

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The actual core loading is determined by assaying the microcapsules by the procedure described above. The actual core loading is calculated in the following manner:

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The encapsulating efficiency is the ratio of the actual and theoretical core loadings and is calculated in the following manner:

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III. PHARMACOKINETICS STUDIES OF AGONIST MICROCAPSULES IN RATS

Pharmacokinetics studies were performed involving the microencapsulation of agonist LHRH in DL-PLGs with varying lactide/glycolide ratios. A formulation of a blend of agonist microcapsules prepared with mole ratios of 52:48, 68:32, and 85:15 DL-PLG excipients were used. This blend consisted of appropriate amounts of 3%-loaded agonist microcapsules prepared with 52:48 DL-PLG, 10%-loaded agonist microcapsules prepared with 68:32 DL-PLG, and 8% loaded against microcapsules prepared with 85:15 DL-PLG excipients. The 52:48 DL-PLG component of the blend was designed to deliver agonist during the first month after administration of the microcapsules. The 68:32 DL-PLG component was designed to release the agonist primarily during the second month after administration, and the 85:15 component was designed to release the agonist primarily during the third through sixth months. Overall, the blend was designed to release approximately 50 μg of agonist per day for 180 days.

Studies with the agonist microcapsules were initiated. A total of 80 male rats were used in the studies. Three groups of 20 rats each were administered three agonist microcapsule formulations, and one group of 20 rats (a control group) was administered placebo microcapsules (empty microcapsules). Blood was collected for six months from the animals receiving the prototype six months formulation, the 85:15 formulation, and the placebo microcapsules. Blood was collected for four months from animals treated with the agonist microcapsule formulation prepared with 68:32

DL-PLG. Ten rats from each group were bled on Fridays. Agonist serum levels were determined for all 80 rats during month 1. Thereafter, agonist serum levels were determined only for rats bled on Fridays.

CONCLUSION

The levels of agonist serum were determined using radio-immunoassay (RIA). RIA results from serum samples collected during the test period showed that a constant release of agonist LHRH was released over the six months. Correspondingly, the concentration of testosterone in serum was found to be suppressed to castrate levels during the controlled release of the LHRH from the single injection of similar microcapsules. After approximately six months, when the microcapsules were depleted of their LHRH, the testosterone levels returned to normal.

Table 1 and Figure 1 show the agonist serum levels obtained with the protype six-month agonist microcapsule formulation.

Table 2 shows the agonist serum levels obtained with agonist microcapsules prepared with 85:15 DL-PLG.

Table 3 shows the agonist serum levels obtained with agonist microcapsules prepared with 68:32 DL-PLG.

Table 4 shows the results of the control group study using placebo microcapsules.

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Claims

1. A composition capable of delivering an effective amount of a constant dose of bio-active

molecule at a constant rate to an animal over a preselected, prolonged period of time, comprising a blend of free-flowing microcapsules in which effective amounts of a bioactive molecule are encapsulated in at least two biodegradable and biocompatible copolymer excipients to form first and second microcapsules, each excipient capable of a different rate of release of said molecule therethrough, said composition having a delivery profile wherein the release of said molecule through said second microcapsule begins as the release of said ingredient through said first microcapsule declines.

2. A composition as claimed in claim 1, wherein said copolymer excipients are poly(D,L-lactide-co-glycolide).

3. A composition as claimed in claim 2, wherein said copolymer excipients have mole ratios of lactide to glycolide of 40:60 to 100:0, respectively.

4. A composition as claimed in claim 1, wherein said active ingredient is a peptide.

5. A composition as claimed in claim 4, wherein said peptide is hormonally active.

6. A composition as claimed in claim 4, wherein said peptide is a luteinizing hormone releasing hormone or an analog thereof.

7. A composition as claimed in claim 6, wherein said luteinizing hormone releasing hormone is [D-Trp , des-Gly]-LHRH ethylamide.

8. A composition as claimed in claim 1, wherein said bioactive molecule is a protein.

9. A composition as claimed in claim 1, wherein said blend of microencapsulated peptide is

comprised of appropriate amounts of 3% by weight loaded [D-Trp , des-Gly]-LHRH ethylamide encapsulated in a copolymer excipient having a mole ratio of 52% lactide to 48% glycolide, and 10% weight loaded [D-Trp , des-Gly]-LHRH ethylamide encapsulated in a copolymer excipient having a mole ratio of 68% lactide to 32% glycolide.

10. A composition as claimed in claim 9 and further comprising an appropriate amount of 8% by weight loaded [D-Trp ,des-Gly]-LHRH ethylamide encapsulated in a copolymer excipient having a mole ratio of 85% lactide to 15% glycolide added to said blend.

11. A composition as claimed in claim 10, wherein said blend delivers [D-Trp ,des-Gly]-LHRH ethylamide at a constant rate of 50 μg to 250 μg per day for 180 days.

12. A composition as claimed in claim 4, wherein said peptide is [D-N-Ac-4-Cl-Phe^2,D-Trp ,D-Ala]-LHRH or an LHRH antagonist analog.

13. A composition as claimed in claim 12, wherein said blend delivers [D-N-Ac-4-Cl-Phe^2,D-Trp ,D-Ala]-LHRH or LHRH antagonist analog at a constant rate of about 200 μg per day for at least 90 days.

14. A method of preparing a composition according to claim 1 for delivering an effective amount of constant dose of a bioactive molecule to an animal over a preselected, prolonged period of time, comprising the steps of:

(a) encapsulating effective amounts of said bioactive molecule in first and second separate biogradable and biocompatible copolymer excipients to form first and second microcapsules, each of said microcapsules capable of a different rate of release therefrom of said molecule; and

(b) combining an effective amount of said first and second microcapsules to form said composition having a delivery profile wherein the diffusion of said molecule through said second microcapsule begins as the release of said molecule through said first microcapsule declines.

15. The method as claimed in claim 14, wherein said first and second copolymer excipients have different monomer ratios.

16. A method as claimed in claim 14, wherein said excipient is selected from the group consisting of polyacetal polymers, polyorthoesters, polyesteramides, polycaprolactone and copolymers thereof, polycarbonates, polyhydroxybutyrate and copolymers thereof, polymaleamides, copolyoxalates, and polysaccharides.